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E. coli F583 Rd2 LPS



**Supplemental Figure 1.** Characterization of Rd2 LPS from *E. coli* F583. (**A**) MALDI mass spectrum of Rd2 LPS from *E. coli* F583 with m/z values of the main peaks indicated. (**B**) Zinc-imidazole-stained SDS-PAGE gel with different amounts of Rd2 LPS from *E. coli* F583 applied to lanes as indicated.



**Supplemental Figure 2**. Chemical shift perturbations in <sup>15</sup>N-<sup>1</sup>H TROSY spectra upon addition of Rd2 LPS from *E. coli* F583 to <sup>2</sup>H-,<sup>13</sup>C-,<sup>15</sup>N-labeled OprH $\Delta$ L1 $\Delta$ L4 in DHPC micelles. (**A**) <sup>15</sup>N-<sup>1</sup>H TROSY spectrum of 0.2 mM OprH $\Delta$ L1 $\Delta$ L4 (black) in DHPC micelles overlaid onto the spectrum of OprH $\Delta$ L1 $\Delta$ L4 in DHPC:Rd2 LPS mixed micelles (red, 10:1 Rd2 LPS:OprH molar ratio). (**B**) Compound chemical shift changes  $\Delta \delta_{comp} = [\Delta \delta^2_{HN} + (\Delta \delta_N / 6.5)^2]^{1/2}$ ) resulting from the addition of 2 mM Rd2 LPS (black) relative to the chemical shifts of 0.2 mM OprH $\Delta$ L1 $\Delta$ L4 in DHPC only. Unassigned residues are marked with blue ticks, and removed loop residues are marked with green ticks.



**Supplemental Figure 3**. Clustal Omega alignment of OprH from *P. aeruginosa* and other closely related outer membrane proteins. Absolutely conserved residues are marked in the bottom line with "\*", highly conserved residues with ":", and partially conserved with "". Absolutely conserved Lys and Arg residues localized in loops 2 and 3 and in strand 7 are highlighted in red. β-strands are indicated with arrows above the sequences. Residue R142, although conserved, is not highlighted because it is facing the lumen of the barrel and therefore cannot interact with LPS.



**Supplemental Figure 4.** <sup>15</sup>N-<sup>1</sup>H TROSY spectra of <sup>15</sup>N-labeled OprH, OprH-R72Q, OprH-R72Q/K103Q and OprH-K70Q/R72Q/K103Q in DHPC micelles (black), overlaid onto the spectra of corresponding proteins in DHPC:Kdo2-lipid A mixed micelles at 10:1 Kdo2-lipid A:protein molar ratio (red).